

Feb. 20, 2003



[0254] Still another aspect of the present invention relates to methods for generating non-human animals and stem cells having a functionally disrupted endogenous gene. In a preferred embodiment, the method comprises the steps of:

- ✓ [0255] (i) constructing a transgene construct including (a) a recombination region having at least a portion of an IBD gene, which recombination region directs recombination of the transgene with the gene, and (b) a marker sequence which provides a detectable signal for identifying the presence of the transgene in a cell;
- ✓ [0256] (ii) transferring the transgene into stem cells of a non-human animal;
- ✓ [0257] (iii) selecting stem cells having a correctly targeted homologous recombination between the transgene and the gene;
- ✓ [0258] (iv) transferring cells identified in step (iii) into a non-human blastocyst and implanting the resulting chimeric blastocyst into a non-human female; and
- ✓ [0259] (v) collecting offspring harboring an endogenous gene allele having the correctly targeted recombination.

✓ [0260] Yet another aspect of the invention provides a method for evaluating the potential of an agent to cause an IBD or to protect against development of an IBD by (i)

contacting a transgenic animal of the present invention with a test agent, and (ii) ascertaining the presence, and more preferably the level, of onset or degree of severity of an inflammatory bowel disease or disorder, and comparing that with an untreated transgenic animal or transgenic animal treated with a control agent.

✓ [0261] X. Exemplification

✓ [0262] The following Table 1 teaches genes whose up-regulation or down-regulation, as indicated by "↑" and "↓", respectively, has been found to be associated with UC and CD. The genes are grouped according to their general functionality, as follows,

- ✓ [0263] I Chemokines+cytokines and growth factors
- ✓ [0264] II Inflammatory mediators
- ✓ [0265] III Cell cycle regulators/transcription factors
- ✓ [0266] IV. Cancer Related
- ✓ [0267] V HLA or immune function genes
- ✓ [0268] VI Antimicrobial
- ✓ [0269] VII ECM and remodelling
- ✓ [0270] VIII Others: Carbohydrate metabolism, Fatty acid metabolism, Protein folding/modification/degradation

TABLE 1

UC	CD	Acc No.	Gene Names	Chromosome	Microsatellite	
					PTO	Markers
I	↑21.4	↑12.8 Y001757	MDNCF/IL-8	4q13-q21	D4S392-D4S2947	
I	↑15.3	X54489	MGSA (GRO1)	4q21	D4S400-D4S1534	
I	↑7.9	M57731	MIP-2 (GRO2)	4q21	D4S392-D4S2947	
I	↑8.9	↑4.1 M28130	IL8	4q13-q21	D4S392-D4S2947	
I	↑6.8	↑3.9 X57251	IP-10	11	pTEL-D11S1318	
I	↑6	J04130	MIP-1 /SCYA4	17q21	D17S933-D17S800	
I	↑3.4	X55800	MIP-2 (GRO3)	4q21	D4S400-D4S1534	
I	↑3.2	M69203	MIP-1 /SCYA2	17q21	D17S933-D17S800	
I	↑4.6	X04500	pro-IL-1	2q14	D2S293-D2S121	
I	↑3.5	X53296	IL-1RA	2q14	D2S293-D2S121	
I	↑3.3	X04602	IL-6	7q21	D7S829-D7S673	
I	↑3	J03756	Growth hormone 2 (GH2)	17q21-q24	D17S794-D17S795	
I	↑3.5	D16431	Ripetoma-derived growth factor (RDGF)	17q2-q24	D17S794-D17S795	
I	↑4	M58286	TNF Receptor member 1A	12p13.2	D12S99-D12S358	
II	↑35.5	S75256	Neutrophil lipocalin (HNL)	—	—	
II	↑10.4	X99133	Neutrophil gelatinase-associated lipocalin (NGAL)	9q34	D9S1821-D9S159	
II	↑8.7	X85781	Nitric oxide synthase (NOS2)	—	—	
II	↑5.1	X65965	Mitochondrial superoxide dismutase (SOD2)	6q25.3	D6S442-D6S1581	
II	↑5.5	14.6 M44230	Phospholipase A2, group IIA (PLA2G2A)	1p35	—	
II	↑5.3	X51441	Serum amyloid A (SAA)	11p	—	
II	↑3.9	J03474	Serum amyloid A (SAA1)	11p15.1	D11S921-D11S1369	

TABLE 1-continued

	UC	CD	Acc No.	Gene Names	Chromosome	Microsatellite Markers
V	13	16	M84526	Complement factor D (DF)	—	pTEL-D19S413
V	13.9		M38690	CD9 antigen	12p13	D12S99-D12S358
V	15		M28590	MHC D _q	6	—
VI	120.4	140.8	M97925	Defensin 5 (DEFA5)	8pter-p21	D8S552-D8S549
VI	16.8	17.7	U33317	Defensin 6 (DEFA6)	8pter-p21	D8S277-D8S550
VII	116.2	13.3	L23808	MMP-12 (Macrophage elastase)	11q22.2-q22.3	D11S1339-D11S1343
VII	16.4		J05070	MMP-9 (Gelatinase B)	20q11.2-q13.1	D20S119-D20S197
VII	14.7		X54925	MMP-1 (Interstitial collagenase)	11q22.3	D11S1339-D11S1343
VII	14.2		X05232	MMP-3 (Stromelysin 1)	11q22.3	D11S1339-D11S1343
VII	113.3	13.8	L10343	Elastase specific inhibitor (Elafin)	20q12-q13	D20S119-D20S197
VII	111	13.1	Z74616	COL1A2	2q37	D2S2158-D2S125
VII	17.3		X52032	COL6A3	2q37	D2S2158-D2S125
VII	16.9	13.6	M55998	COL1A1	17q21.3-q22	D17S791-D17S794
VII	14.8		X06700	COL3A1	2q31	D2S2257-D2S115
VII	14.7		X15882	COL6A2	21q22.3	—
VII	13.9		X05610	COL4A2	13q34	D13S285-qTEL
VII	13.7	13.3	HG2157-HT2227	Mucin 4 (MUC4)	3q29	—
VII	13.1		X52003	Trefoil factor 1 (TFF1)	21q22.3	D21S1259-qTEL
VII	14.6	M22406		Intestinal mucin	—	—
VII	16.4	J03040		Osteonectin (SPARC)	5q31.3-q32	D5S436-D5S470
VII	14	13.2	X17042	Proteoglycan 1 (PRG1)	10q22.1	D10S210-D10S537
VII	13.9		D11428	Peripheral myelin protein 22 (PMP22)	17p12-p11.2	D17S804-D17S799
VII	13.8		X02761	Fibronectin 1 (FN1)	2q34	D2S137-D2S164
VII	13.7		M77349	Transforming growth factor beta-induced (TGF β)	5q31	D5S393-D5S500
VII	13.2		D13666	Osteoblast specific factor 2 (OSF-2)	13	D13S267-D13S1253
VII	13.1		M10321	von Willebrand factor	12p13.3	D12S99-D12S358
VII	13		L09190	Trichohyalin (THH)	1q21-q23	D1S439-D1S459
VII	13.1	D88422		Cystatin A (CSTA)	3q31	—
VII	14.7	X58199		Adducin 2 (ADD2)	2p13-p14	—
VII	13.7	MS6933		Amelogenin (AMELY)	Yp11.2	—
VII	13.2	D45370		Adipose specific collagen-like 2 (APM2)	10	D10S1786-D10S541
VII	13.8	X73501		Cytokeratin 20	—	—
VII	14	U60061		Zygfin 2	2	D2S367-D2S2230-D2S177-D2S119
VII	13.1	AF006087		Actin-related complex	3	D3S3591-D3S1283
VII	13.6	D87460		Paniemmin	19p13.3	pTEL-D19S413
VIII	150.5	D26416		Esterase D (ESD)	15q14.1-q14.2	D13S328-D13S168
VIII	14.7		M15656	Aldolase B	9q21.3-q22.2	D15S202-D15S157

TABLE 1-continued

UC	CD	Acc No.	Gene Names	Chromosome	Microsatellite Markers
VIII	†6.3	J04040	Glucagon (GCG)	2q36-q37	D2S156-D2S376
VIII	†4.4	L31801	Monocarboxylate transporter 1 (MCT1)	1p13.2-p12	D1S418-D1S514
VIII	†3	D10523	Oxoglutarate dehydrogenase (OGDH)	7p14-p13	D7S521-D7S478
VIII	†4	M12963	Alcohol dehydrogenase 1a (ADH1)	4q21-q23	—
VIII	†4.5	Y00339	Carbonic anhydrase II (CA2)	8q22	D8S275-D8S273
VIII	†4.9	†3.1 L10955	Carbonic anhydrase IV (CA4)	17q23	—
VIII	†12.7	†3.1 L05144	Phosphoenolpyruvate carboxykinase 1, soluble (PCK1)	20q13.31	D20S183-D20S173
VIII	†3	U07158	Syntaxin 4A (STX4A)	—	—
VIII	†3	— L27706	Chaperonin subunit 6A (CCT6A)	7	D7S530-D7S509
VIII	†7	†3.1 J04093	UDP-glycosyltransferase 1 (UGT1)	2	D2S2158-D2S125
VIII	†3.2	U20499	Sulfotransferase family 1A (SULT1A3)	16p11.2	—
VIII	†3	M15182	β-glucuronidase (GUSB)	7q21.11	—
VIII	†4	U08854	UDP glucuronosyltransferase precursor (UGT2B15)	4q13	D4S1619-D4S392
VIII	†5	D87292	Thiosulfate sulfurtransferase (TST)	22	D22S277-D22S283
VIII	†3	†4 M22324	Aminopeptidase N/CD13 (ANPEP)	15q25-q26	D15S202-D15S157
VIII	†12	†7 M22960	Protective protein for β-galactosidase (PPGB)	20q13.1	D20S119-D20S197
VIII	†3.4	X90908	Fatty acid binding protein 6 (FABP6)	5q23-q35	—
VIII	†4.1	J02874	Fatty acid binding protein 4 (FABP4)	8q21	—
VIII	†3	M10050	Fatty acid binding protein 1 (FABP1)	11p15.5	D11S1318-D11S909
VIII	†3	L24774	Mitochondrial d3, d2-CoA-isomerase	—	D
VIII	†4	D16294	Mitochondrial 3-oxoacyl-CoA thiolese (ACAA2)	18	— D18S1118-D18S474
VIII	†4	M77144	3 β-hydroxysteroid dehydrogenase (HSD17B2)	1p13.1	D1S418-D1S514
VIII	†5	D10511	Mitochondrial acetoacetyl-CoA thiolese	—	—
VIII	†7	Z80345	Acyl-Coenzyme A dehydrogenase (ACADS)	12q22-pter	D12S366-D12S340
VIII	†7	L11708	17 β-hydroxysteroid dehydrogenase II (HSD17B2)	16q24.1-q24.2	D16S515-D16S422